ISSN: 0975 – 8712
IJFSNPHT (2018), 10(3):18-25

DOI: http://doi.org/10.5281/zenodo.1488388

Bacterial Load of Fresh Vegetables Sold in a Selected Public Market and their Susceptibility to Commonly Used Antibiotics

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ABSTRACT:
The World Health Organization (WHO) has ranked green leafy vegetables as one of the highest priorities among fresh vegetables in terms of microbiological hazards. The study aimed to identify the different microorganisms which can be found on fresh vegetables sold in a selected public market and their susceptibility to commonly used antibiotics. To assess their microbial quality, five lettuce and five cucumber samples each weighing two hundred fifty grams were collected and washed. The washings load for lettuce ranged from 2.50 × 104 – 2.41 × 106 cfu/ml while for cucumber it ranged from 7.00 × 103 – 3.67 × 104 cfu/ml. Enterobacter cloacae (35%) was identified as the most prevalent bacterial species from the samples followed by the following species: Pantoea spp. (22%), Klebsiella pneumonia (14%), and Citrobacter freundii (7%). The antibiotic resistance of isolated organisms was also determined Citrobacter freundii, Enterobacter asburiae, Enterobacter cloacae and Enterobacter sakazakii were susceptible to augmentin, impenem, and ampicillin while Klebsiella oxytoca and Klebsiella pneumonia spp showed resistance to augmentin and ampicillin. The bacterial load of all the vegetables tested has exceeded the accepted values set by National Standards of Iran in assessing microbiologic quality of certain fresh vegetables. All organisms isolated from the samples belong to Family Enterobacteriaceae. Most of the isolates showed resistance to ampicillin, augmentin, and imipenem.

Key words: Bacterial Load, Fresh Vegetables, Susceptibility, Antibiotics

INTRODUCTION

The World Health Organization (WHO) has ranked leafy green vegetables as one of the highest priority among fresh fruits and vegetables in terms of microbiological hazards [1]. It has been reported that leafy green vegetables are responsible for a number of outbreaks of foodborne illness worldwide. Fresh vegetables carry with them, on the surface, abundance of pathogenic microorganisms that may come from several factors such as farm locations, storage temperature or time and transport conditions. As most of these vegetables are eaten without further processing or eaten raw, its microbial content could be a big risk factor to the consumer's health condition for it may bring various foodborne illnesses. Contamination can arise as a consequence of treating soil with variety of fertilizers, such as sewage sludge and manure, and from the irrigation water, as well as from the ability of pathogens to persist and proliferate in vegetables. Indirect contamination, on the other hand, may also occur as a result of poor hygienic environment, poor handling or washing.

Serious health threat due to the presence of pathogenic microbes in food can lead to food poisoning and even immediate death to those who are unfortunate. Enteric pathogens Salmonella and *Escherichia coli* (E.coli)

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O157:H7 are the majority of the foodborne illness associated with leafy vegetables globally. Listeria monocytogenes was also recorded as the primary pathogen of concern in ready-to-eat food because of its ability to grow under refrigeration temperatures, as according to Food and Safety Action Plan (FSAP) of Canadian Food Inspection Agency [2].

The most common clinical presentation of foodborne disease takes the form of gastrointestinal symptoms; however, such disease can also have neurological, gynecological, immunological and other symptoms. Multiorgan failure may result from the ingestion of contaminated foodstuffs, thus representing a considerable burden of disability as well as mortality.

According to Center for Disease Control (CDC) [3], each year 76 million people get sick, more than 300,000 are hospitalized and 5,000 die as a result of foodborne illness. This study aimed to identify the different organisms which can be found on fresh vegetables that are sold in selected public markets and their susceptibility to commonly used antibiotics.

MATERIALS AND METHODS

1.Design and Sampling Technique

The study used the cross-sectional method of research which involved the isolation of bacteria, its characterization and the determination of microbial load and their susceptibility to specific antibiotics. Vegetables tested in the study were obtained from all stalls in a selected public market that are both selling cucumber and lettuce. While there may be vegetable stalls outside the market, all samples that were tested in the study were obtained only from registered stalls that were selected through purposive sampling.

2. Collection of Samples

Two types of vegetable samples that are commonly eaten raw namely lettuce (*Latuca sativa*) and cucumber (*Cucumis sativus*) were collected from Trabajo Market located at Sampaloc, Manila. Stalls selling both lettuce and cucumber were only included. A total of ten samples, five lettuces and five cucumbers, weighing two hundred fifty grams each were collected from five randomly selected stalls and placed in a sterile plastic bag which contained two hundred fifty millimeter (250 mL) phosphate buffer to maintain the viability of the microorganisms transported.

3. Microbial Analysis and Isolation

All the samples were rinsed thoroughly using phosphate buffer solution. 6-fold serial dilutions of each washing were made. One mL of the different dilutions was pipetted into sterile petri dishes with Nutrient Agar and mixed through and through to allow the distribution. The plates were allowed to solidify, and incubated at 37°C for 24-48 hours for colony formation. Followed by appropriate incubation period, all plates were examined for colonial growth. Colony Forming Unit/ml was determined by counting all colonies using a colony counter and to calculate, this formula was used: CFU = 1/Average colony count × dilution factor × volume used [4]. Three trials were carried out from each dilution.

To establish comparison with the isolates to be obtained, commercially available *Escherichia coli* ATCC 35018 was used. A Mueller Hinton agar was seeded with control organisms. Antibiotic disc containing Ampicillin, Augmentin, Imipenem and Ceftriaxone were used. After incubation at 37°C for 24-48 hours, the diameter of each zone was measured using a Vernier calliper that evaluated susceptibility or resistance. Results obtained from the control

and from the isolates should met the established that validated the results.

In the isolation of enteric organisms, washings from the different vegetables samples were inoculated on Mac Conkey Agar. The said media is a selective and differential media used to isolate and differentiate non-fastidious gram negative organisms, particularly members of the family *Enterobacteriaceae* and Genus *Pseudomanas.* The inclusion of crystal violet and the bile salts in the media prevents the growth of the gram positive bacteria and fastidious gram negative bacteria such as *Neisseria* and *Pasteurella.* Neutral red is the incorporated pH indicator. Carbohydrate and peptone content of the medium are used to enhance the growth of enteric pathogens.

Lactose fermenting organisms produce pink to red colonies on the said media, while those that do not ferment lactose will appear colorless on the media. All inoculated plates were incubated at 37°C for 24-48 hours.

4. Phenotypic Characterization of Isolates

Further test was carried out to identify all isolated organisms. The Analytical Profile Index (API) Kit was utilized for the identification. The API 20 E is a miniaturized panel of biochemical tests compiled for identification of groups of closely related antibacterial agents were placed on the inoculated Mueller Hinton Agar plates and were incubated for 37°C for 24-48 hours.

This test was performed against the following antibiotics: Ampicillin, Augmentin, Imipenem, and Ceftriaxone. After 24 hours, the zone of inhibition was measured using Vernier caliper. The presence or absence of growth around the disks determined the ability of the antimicrobial agent to inhibit the organism. bacteria. It uses test kits for the identification of Gram positive and Gram negative bacteria and yeast.

With multiple biochemical reactions in limited wells along with a wide identification database on the market, API makes bacterial identification reliable and accurate. The kit enables the identification of 700 species of bacteria and yeasts, has a complete report for each identification including proposed species, and a complete biochemical profile. Different test panels are prepared in dehydrated forms which are reconstituted upon use by addition of bacterial suspensions. After incubation, positive test results are scored as a seven-digit number (profile). Identify of the bacterium is then easily derived from the database with the relevant cumulative profile code book or software.

5. Antibiotic Susceptibility

The antibiotic resistance and sensitivity of the isolated bacteria in lettuce and cucumber were tested using the disc diffusion method using commercially available antibiotic disc. Kirby-Bauer Disk Diffusion Method was used that determined the susceptibility or resistance of the isolated organisms to different antimicrobials. The isolated organisms were inoculated in Mueller Hinton Agar using the overlap streaking method. The disc containing The antimicrobial susceptibility test results of all isolated organisms were compared using the Clinical and Laboratory Standards Institute (CLSI) [5].

RESULTS AND DISCUSSION

1. Bacterial Load

Bacterial load may be defined as the measurable quantity of bacteria in object. Mirrored in Table 1 is the colony count of each

ISSN: 0975 – 8712
IJFSNPHT (2018), 10(3):18-25

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lettuce and cucumber samples that were analyzed.

Based on the results, lettuce samples exhibited to have higher colony count than the cucumber samples. This is in agreement with the research conducted by Itohan et al. [6] where a total of fifteen vegetables samples (carrots, lettuce, cucumber) collected from different markets in Nigeria were evaluated for bacterial load. In their study, lettuce samples tested gave the highest bacterial load which is 2.9 × 108 cfu/g (2010). They also reported that the abundance of bacteria on the lettuce samples can be attributed to large surface area of the leaves suitable for water contact, making them more susceptible to bacterial contamination.

Unlike lettuce, cucumber is not often contaminated by soil pathogens as it does not come in contact with soil. In addition, contamination of vegetables with pathogens could gas a result of poor processing methods which may involve spraying the vegetables with water. Although spraying with water gives fresh appearance to the vegetables, and delays decomposition it could add microorganisms. Spraying vegetables with water provides moist surface that encourages bacterial growth on longer storage. Containers used during transport unless adequately sanitized may likewise subject vegetables to contamination.

As shown in Table 1, lettuce sample no. 1 gave the highest count of 2.41 × 106/mL, followed by the lettuce samples no. 5 (8.03 × 105/mL). While cucumber sample no.1 gave the highest count (3.67 × 104/mL), cucumber sample no. 2 was recorded to be least contaminated (7.0 × 103/mL).

The high bacterial load in vegetable salad ingredients can be considered as an indicator to promote awareness on the health hazards that could result from poor handling of vegetables [6]. It was also found out that vegetables might have an important role as a source of multiple antibiotic resistant bacteria especially those that are sold in an open market [7, 8].

Currently there is no available data provided by the Philippine Food and Drug Administration, Department of Agriculture, or the Department of Trade and Industry – Philippine National Standards to determine the acceptable bacterial load for fresh vegetables. However, based on the National Standards set by Iran in determining microbiological quality of food which was used as basis in the study of Jeddi et al. [9], only lettuce sample 1 does not conform to the accepted microbial load which is 1.0 × 106CFU/mL.

Table 1 Bacterial Load of Vegetable Samples

Lettuce Samples	Colony Count (CFU/mL)	Cucumber Samples	Colony Count (CFU/mL)
1	2.41×10^6	1	3.67×10^4
2	5.00×10^5	2	7.00×10^3
3	3.37×10^5	3	7.33×10^3
4	2.50×10^4	4	1.47×10^4
5	8.03×10^5	5	9.00×10^3

ISSN: 0975 – 8712
IJFSNPHT (2018), 10(3):18-25

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2. Species of Bacterial Isolates from Lettuce and Cucumber

Depicted in Figure 1 are the percentages of organisms isolated from lettuce and cucumber samples. As shown in the graph, Enterobacter cloacae was noted as the most prevalent species (36%) while Citrobacter freundii was identified as the least predominant organism isolated form the vegetables (7%). Other Enterobacter spp., Klebsiella spp. and Pantoea spp. were also found to contaminate the samples.

Although the findings of the study contradict that of Akinyele et al. [10] Murtaza et al. [11] in which Staphylococcus aureus showed the highest occurrence in most of the vegetable tested, results of the present study is consistent with the findings of Falomir et al. [12] and in which Enterobacter cloacae was also identified as the most predominant organism in fresh vegetables.

Enterobacter cloacae which is usually considered part of the normal flora, is an opportunistic pathogen causing infection in immunocompromised patients if suitable opportunity arises. Although cases of *Enterobacter cloacae* infection occur as a nosocomial infection, the results of the present study suggest that it can also be a potential pathogen in marketed fresh produce like lettuce and cucumber which are commonly eaten raw and as such can serve as a risk factor for consumer's health.

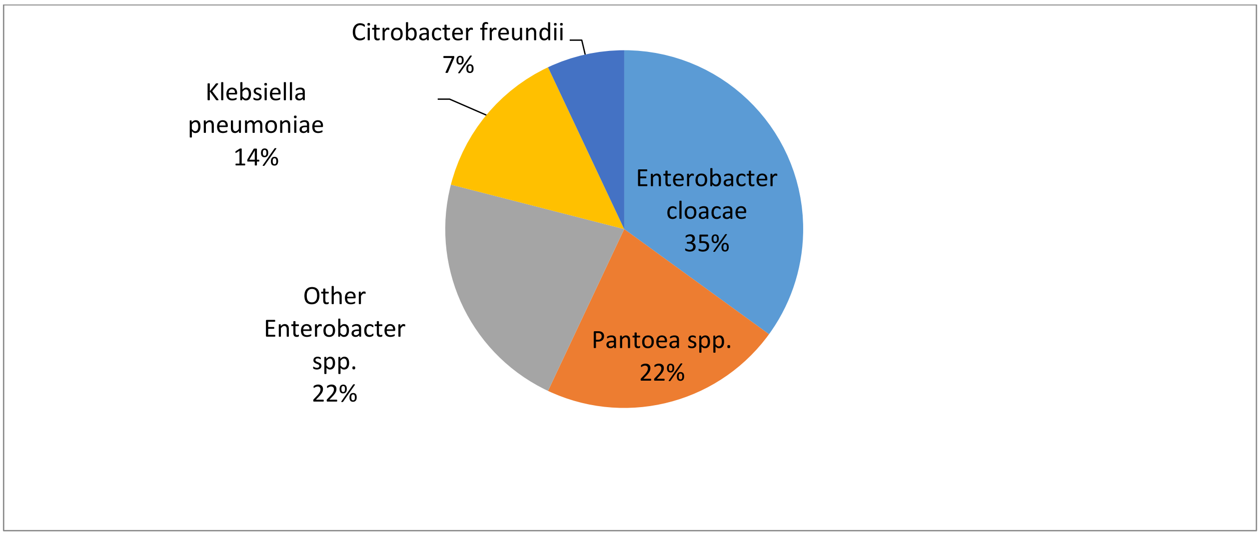

Figure 1. Percentages of Bacterial Isolates from Vegetable Samples

3. Antimicrobial Susceptibility Testing

Antibiotic susceptibility test was done to determine resistance of the isolated organisms to the following antibiotics commonly used for treatment of intestinal infection. These include

ISSN: 0975 – 8712
IJFSNPHT (2018), 10(3):18-25
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the following: Augmentin, Ampicillin, Ceftriaxone and Imipenem.

As revealed in Table 2, *Citrobacter freundii, Enterobacter absuriae, Enterobacter cloacae, Enterobacter sakazakii* were resistant to most antibiotics used in the study. Kim et al. [13] and Gozalbo et al. [14] reported the same results in the previous studies they conducted.

While *Pantoea spp.* 2 and *Pantoea spp.* 4 showed resistance to Ampicillin, only *Enterobacter aerogens* exhibited susceptibility to Augmentin, Ampicillin, Ceftriaxone and Imipenem, the results suggest that isolates exhibiting multi resistance to chemotherapeutic agents can be a potential carrier of resistance from farm to consumers which may constitute and additional food safety concern.

Table 2 Antibiotic Resistance of Isolated Organisms

Isolate	Antibiotic Resistance
Citrobacter freundii	Augmentin, Imipenem, Ampicillin
Enterobacter aerogenes	None
Enterobacter asburiae	Augmentin, Imipenem, Ampicillin
Enterobacter cloacae	Augmentin, Imipenem, Ampicillin
Enterobacter sakazakii	Augmentin, Imipenem, Ampicillin
Klebsiella oxytoca	Augmentin, Ampicillin
Klebsiella pneumoniae spp pneumonia	Augmentin, Ampicillin
Pantoea spp 2	Ampicillin
Pantoea spp 4	Ampicillin
Escherichia coli 1 (Control)	Augmentin, Ampicillin

It can be noted that ampicillin showed the highest resistance rate in the bacteria isolated from the samples. Similar finding were also noted in several studies showing the highest resistance rate in ampicillin in the vegetables collected in the market. [15, 16, 17, 18]

CONCLUSION

Among the samples, only the bacterial load of lettuce sample 1 has exceeded the accepted values set by the National Standards of Iran in assessing microbiologic quality of certain fresh vegetables and that most of the organisms isolated from the samples belong to Family *Enterobacteriaceae*. Furthermore, most of the isolates showed resistance to Ampicillin, Augmentin, and Imipenem.

RECOMMENDATIONS

In view of the aforementioned findings and the conclusion, the recommendations proposed includes initiation of government-led information campaign that will focus on the proper use of manure to prevent potential risk of contaminating fresh produce, encouraging the consumers to observe sanitary practices like thorough washing of vegetables to ensure that goods will be bacteria-free prior to consumption and conducting similar studies using larger number of samples, covering more markets, identification of gram positive organisms and testing susceptibility and resistance of organisms to other antimicrobial agents.

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